Abstract

A simple and direct method for the simultaneous correction of steady-state polarized fluorescence intensities, depolarized (or scrambled) by the effects of applied hydrostatic pressure is described. In the method discussed here, it is not necessary to first determine the scrambling factors from a separate experiment with a dye immobilized in a rigid medium. Rather correction for depolarizing effects of the high pressure spectroscopy cell windows is achieved by direct recalculation of the measured polarized data obtained for the sample of interest at the time of data collection. This method of correction is tested for common fluorescent dyes 1,6-diphenyl-1,3,5-hexatriene (DPH) and 9,10-diphenylanthracene (DPA) in glycerol where their rotational behavior is well understood. In addition, the pressure induced 'melt' profile for the more complicated biologically relevant system of DPH imbedded within dipalmitoylphosphatidylcholine (DPPC) small unilamellar vesicles (SUVs), has been reexamined. While the method discussed here is used for the correction of steady-state polarized data, it may be easily adapted for use in time-resolved polarized fluorescence measurements. Advantages and limitations of the new correction method are disclosed.